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Use of Daily Growth Increments on Otoliths to Assess Stockings of Hatchery-Reared Kokanees

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Abstract.- -Hatchery-reared kokanees *Oncorhynchus nerka* may form discernible "stress" checks on their otoliths when they are stocked into lakes and rivers. We established the reliability of this mark for distinguishing age-0 hatchery fish from wild fish in Lake Pend Oreille, Idaho. Hatchery fish marked with dietary oxytetracycline (OTC) were recaptured 1-3 months after they had been stocked in the lake. All specimens with OTC marks on their bony structures also had a stress check. We also validated daily otolith increments for age-0 kokanees by comparing increment counts external to the stress check with known days between release and recapture of hatchery fish. Then, counts of daily increments allowed us to correctly identify fish from several co-occurring release groups that had been stocked at different times in the same season. Such discrimination will enhance evaluations of alternative stocking strategies. Because some otolith growth increment counts varied slightly from days at large, and because fish released in cold, food-poor, or otherwise unfavorable sites apparently did not resume otolith growth immediately, we recommend that groups of kokanee be released at least 7 d apart if they are to be subsequently identified by otolith analysis.

Since Pannella (1971) first documented daily growth increments in fish otoliths, the phenomenon has been validated for many species of fish (see review by Campana and Neilson 1985), including sockeye salmon *Oncorhynchus nerka* (Marshall and Parker 1982; Wilson and Larkin 1982). Deposition of calcium on the otolith surface can be inhibited, leaving a discernible check mark, if a fish is subjected to a lower temperature or other form of stress (Brothers and McFarland 1981; Campana 1983). Such checks can be induced in otoliths of hatchery-reared fish, where they form reference marks for subsequent studies of age and growth based on daily otolith growth increments (Campana and Neilson 1985).

We applied these principles to stocking evaluations of kokanee, the nonanadromous form of sockeye salmon. Kokanees are important sport and food fish in Idaho (Simpson and Wallace 1982), and efforts are underway to restore kokanee populations in some Idaho waters through releases of hatchery-reared fish. Assessment of these stockings at Lake Pend Oreille is important because 7-13 million fry were released annually at several locations and at different times of the year. An ability to identify and quantify the survivors of each release group, as they appear in subsequent trawl catches, would enable us to determine the best stocking strategy. The objectives of the study reported here were (1) to determine if a stocking stress mark exists on otoliths of kokanee fry released into Lake Pend Oreille, (2) to learn if such a mark can be used reliably to distinguish hatchery fry from wild fry, (3) to validate a daily periodicity of otolith increments for kokanee fry released in the lake, and (4) to determine if groups of fry released at various times could be identified subsequently from counts of otolith increments. All objectives were achieved.

Methods

The study was conducted at Lake Pend Oreille, a deep, oligotrophic, 37,700-hectare glacial lake in northern Idaho (Figure 1). The Clark Fork River is its main tributary and the Pend Oreille River is its outlet.

Kokanee eggs were obtained during spawning runs in tributaries of the lake. They were fertilized and moved into the Cabinet Gorge Fish Hatchery on the Clark Fork River (Figure 1), where fry were reared to lengths of about 50 mm for stocking.

Three to six fry release strategies-combinations of four release locations (Clark Fork River, Sullivan Springs, open lake, and south lake shoreline) with two stocking periods (May-June and July)--were evaluated each year during 1988-1990. In each year groups of 1.0 to 3.4 million fry

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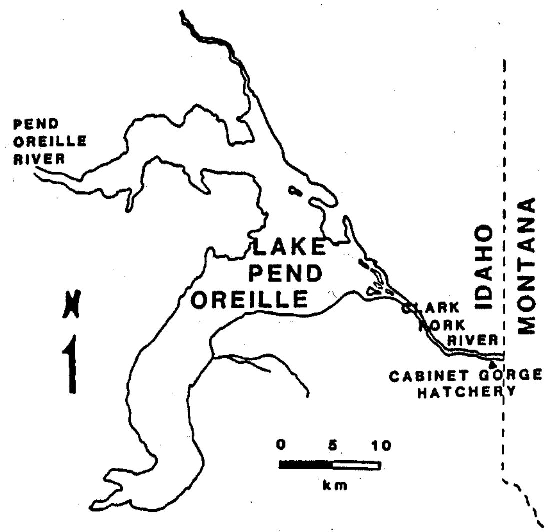

FIGURE 1.--Locality map of Lake Pend Oreille, Idaho.

were released at intervals of at least 1 d and usually more than 4 d.

About 4.5 million fry released in 1988 and 3.3 million in 1989 had been marked with oxytetracycline (OTC, TM-100) administered in their feed during hatchery residence. Oxytetracycline was mixed with fish feed at a rate of 5.5% of diet weight and fed to kokanees for 10 d prior to their release into Lake Pend Oreille. All fry were marked and held inside the hatchery or under covered raceways because the mark degrades when exposed to ultraviolet (UV) light. Age-0 kokanees captured in the late summer by trawl were examined for OTC marks with a long-wave (3,600-angstrom) UV light. When exposed to UV light, a yellow sheen is observed around the mandibles, opercles, and bases of pelvic and pectoral fins. Retention of OTC was tested with four replicated experiments of 500 fish each in 1988 and 1989. Kokanees fed OTC were held in separate raceways and retention was monitored weekly for 10 weeks after feeding. At each weekly interval, 10 kokanees from each raceway were examined for OTC. We found 100% retention by kokanees for the 10 weeks in each year.

Kokanees were collected with a midwater trawl from the lake over a period of five nights during the last week in August or the first week of September of each year, at the time of the new moon. After capture, a UV light was passed over the age-0 kokanees and they were then segregated by our positive or negative observation of a yellow sheen.

In the laboratory, both sagitta otoliths were excised and embedded in a low-viscosity medium (Spurr 1969). The proximal surface was polished with 600 grit paper and the otolith microstructure was observed (1,OOOx magnification) in oil immersion with a compound microscope interfaced with a video camera and monitor. Otoliths were prepared and analyzed by two investigators in 1988 and 1989 and by two others in 1990; the two new analysts were trained by the principal investigator. The analysts looked for a stress check on each otolith. Structures with checks were set aside for later counts of growth increments (the dark members of light-dark band pairs) between the mark and the external surface. The second investigator in each case analyzed a random sample of 50% of the otoliths; in every case identification of the stress check conformed to that of the first investigator. Some differences in daily growth increment counts were found between analysts but seldom differed by more than 2 d. In cases of disagreement, investigators viewed the otoliths together on the monitor and counted increments to resolve each difference.

The reliability of the stress check as an indicator of hatchery origin was assessed from co-occurrences of checks with OTC marks on bony structures of fish captured in 1988 and 1989. The daily periodicity of otolith growth increments was tested by simple regression analysis of band counts with respect to the number of days fry were at large in the lake; this analysis was done by pooling release groups each year. Number of days at large was the difference between median release date and recapture date or the exact number of days at large when a group was released on a single day. If fewer than 20 hatchery fish of a release group were recaptured, the whole sample was analyzed; 20-40 fish were randomly selected from larger recapture samples. Analysis of variance (ANOVA) and Bonferroni pairwise comparisons (Miller 1966) were used to evaluate deviations from daily periodicity in otolith deposition among sites and years. This comparison was restricted to 1988 and 1989, when the same five stocking strategies were used.

Results and Discussion

Stress Checks on Otoliths

All otoliths examined from age-0 kokanees of known hatchery origin in Lake Pend Oreille exhibited a stress check followed by reduced growth increment widths. Trawl samples contained 992 age-0 kokanees in 1988 and 602 in 1989. Of these, 240 had OTC marks, indicating they were of definite hatchery origin. We examined otoliths of 224

OTC marked fish and all had a stress check. The stress checks always occurred where increment widths decreased sharply by half (Figure 2), indicating a sudden decrease in growth rate. We analyzed a random sample of 684 (51 %) otolith pairs from the remaining fish and identified 434 non-OTC hatchery fish by the stress mark and change in increment width. We concluded the remaining 250 fish were of natural origin because they lacked a stress check and had uniform growth increments. Furthermore, no stress checks were observed on otoliths of 25 fry sampled from the hatchery, just before others of the cohort were released. We believe the 100% co-occurrence (N = 224) of OTC marks, stress check, and change in width of growth increments was a valid indicator of hatchery origin. Thus, we conclude that these checks are reliable indicators of hatchery origin and that they form as a result of the stocking process. We call them "release marks" hereafter.

Periodicity of Otolith Increments

The number of otolith increments external to release marks agreed closely with the number of days fish were at large in the lake (Table 1). Over the 3 years, 96% of increment counts differed by 2 or less from days at large. The number of increments exactly matched the actual or estimated days at large for a majority of the fish examined (47% in 1988, 69% in 1989, 71% in 1990). Most of the other fish had one or two increments less than the number of days, a few had greater ring deficits, and a few had one more increment than days at large.

Age-0 kokanees at Lake Pend Oreille produced daily growth increments on a 1:1 relationship to the number of days at large. Simple regression analysis of the 14 means of days at large versus the number of daily growth increments (Table 1) provided a highly significant relationship (r^2 = 0.99, P = 0.000), and ANOVA testing indicated the slope of the regression was equal to 1 (P < 0.001) (Table 2). The 1:1 relationship was graphically demonstrated by superimposing the paired points on a diagonal line with a slope equal to 1; the paired points produced a near-perfect fit (Figure 3). We conclude that age-0 kokanees increment their otoliths daily in Lake Pend Oreille.

Identification of Release Groups

By counting daily otolith increments external to release marks, we successfully discriminated as many as six coexisting release groups of age-0 kokanees collected from Lake Pend Oreille (Figure 4). The only ambiguity was a slight overlap in increment counts between south shore and late open-water groups in 1989; these two groups had been released only 1-2 d apart and were differentiated by the presence or absence of an OTC mark (the late open-water fish had not been marked with OTC). We consider the release mark and daily increment method a sensitive means of identifying hatchery fish to their proper release group in Lake Pend Oreille, provided that releases are temporally separated by several days.

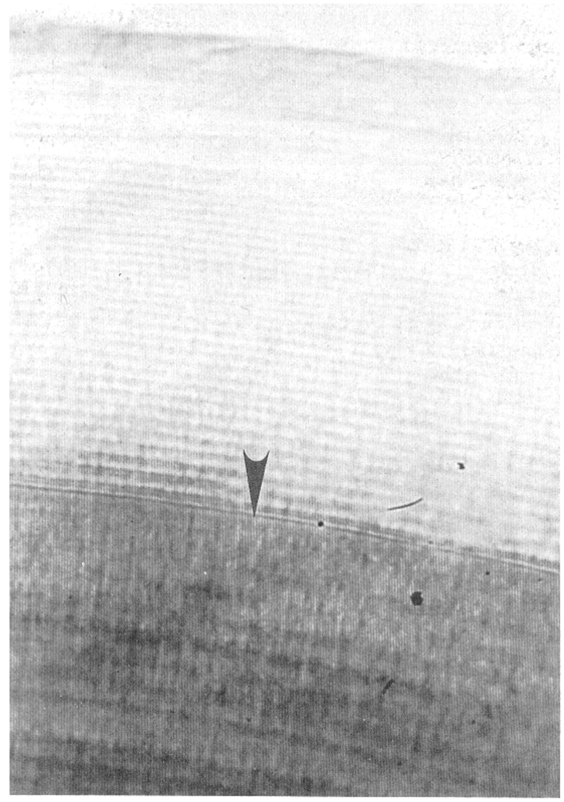

FIGURE 2.-Processed. otolith of an age-0 kokanee captured in Lake Pend Oreille. The arrow marks a stress check and points toward the otolith's centrum.

Spatiotemporal Variations

The number of otolith increments that fish accumulated per unit time at large differed slightly, although significantly (α = 0.10), among stocking groups and years during 1988-1989 (Table 3). Fish released early in Clark Fork River typically developed fewer increments (although they were at large longer; data were adjusted for days at large) than did fish in other release groups (P < 0.06); so, to a lesser extent, did fish released early in the open lake (P < 0.12; Table 4). These release groups encountered the least hospitable conditions: wa-

TABLE 1.--Stocking strategies and otolith growth in relation to time at large for hatchery-reared kokanees released into Lake Pend Oreille, 1988-1990. Mean lengths of fry at stocking varied between 46 and 57 mm among release groups.

Stocking strategy (place, time)	Release		Days at large[a]	Number of otolith increments	
	Number (millions)	Date		Mean (SD)[b]	N^c
1988 (recapture Sep 8-13)					
Clark Fork River					
Early	3.414	Jun 15	86	84.7 (0.92)	20
Late	1.297	Jul 5-9	64	63.9 (0.37)	20
Sullivan Springs	5.139	Jul 11-14	59	58.9 (0.31)	20
Open water					
Early	1.607	May 27	74	73.3 (0.64)	20
Late	1.570	Jul 26	45	43.3 (0.73)	30
1989 (recapture Aug 28-30)					
Clark Fork River					
Early	3.513	Jun 21	69	68.7 (1.09)	30
Late	0.984	Jul 17-19	42	42.5 (0.76)	20
Sullivan Springs	3.538	Jul 11-13	48	48.7 (0.87)	30
Open water					
Early	1.256	Jun 29	61	62.1 (1.46)	7
Late	1.428	Jul 26	37	36.6 (1.16)	30
South Shoreline	1.024	Jul 27-28	34	33.3 (1.21)	30
1990 (recapture Aug 20-24)					
Clark Fork River					
Early	3.430	Jun 26	59	58.7 (0.92)	40
Sullivan Springs	3.190	Jul 10	44	43.6 (1.34)	40
South shoreline	1.140	Jul 29	29	29.8 (1.64)	40

[a] Median recapture date minus median release date.
[b] Increment counts from stress check to outer surface.
[C] Number of recaptured fish examined.

ters were colder and planktonic food was less abundant than at other release times and places, and these fish survived less well than fish in other groups (unpublished departmental data). The lower increment counts probably reflect delayed resumption of growth after stocking. Tzeng and Yu (1992) found that otolith growth increments in starved larval milkfish *Chanos chanos* were not deposited daily. The differences between expected and actual daily growth increment counts in our study were small-less than two increments-but they reinforce the need to separate release groups by several days so that environmental variability will not obscure later group identifications by otolith analysis.

Evaluation of the Method

The release mark and counts of daily growth increments provided several advantages over other marking and identification techniques. One important advantage is that fish do not need to be handled prior to release, as they do with fin clipping, batch-marking with grit or pigments, or coded wire tags. Costly labor is avoided because additional handling is not required and expenses for

TABLE 2.--Simple regression analysis of means of days at large (from release to recapture) versus number of otolith increments external *to* reference marks for age-0 kokanees released in Lake Pend Oreille, 1988-1990.

N	Variable	Coefficient	SE	r^2	Adjusted t	P (two tail)
14	Constant	0.534	0.742	0.998	0.720	0.486
	Days out	0.986	0.013		74.276	0.000

TABLE 3.--Analysis of variance of number of otolith increments for age-0 kokanees stocked at various sites in Lake Pend Oreille, *1988* and 1989. Asterisks denote $P < 0.001$**.

Source	Sum of squares	df	Mean square	F
Site	33.438	4	8.359	20.408**
Year	39.953	1	39.953	97.539**
Site x year	25.712	4	6.428	15.693**
Error	84.790	207	0.410	

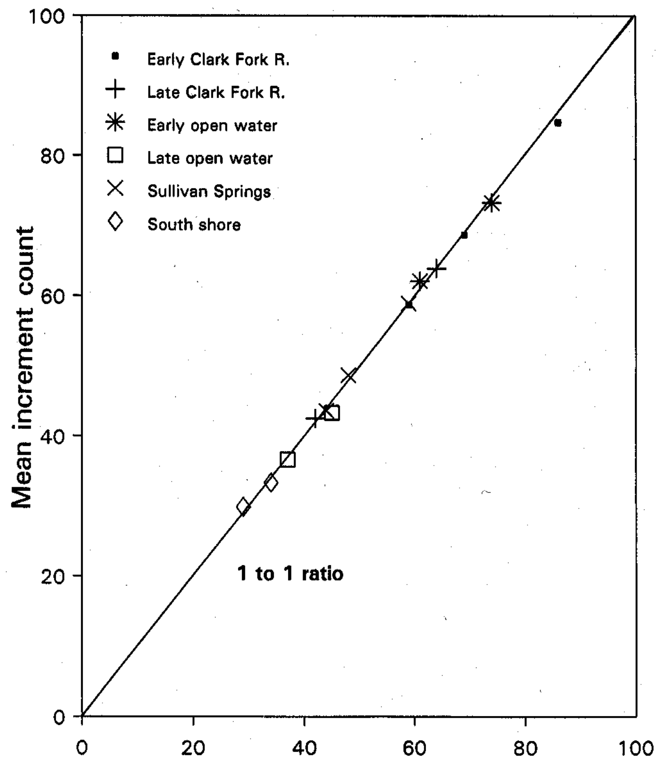

FIGURE 3.--Relationships of otolith increments since formation of release marks to days at large for 14 groups of age-0 kokanee stocked into Lake Pend Oreille, 1988-1990. The diagonal reference line (not fitted to data) represents the 1:1 relationship of increments to days.

chemicals or feed additives are not necessary. Nor is there a concern for the loss of a mark, tag shedding, or regeneration of clipped fins. The laboratory technique is straightforward and does not require extensive training, and the materials needed are commonly available from scientific supply vendors and hardware stores. Also, several releases of fish can be evaluated simultaneously.

Disadvantages of the method include the initial investment in a compound microscope that can give 1,000 X magnification. Although a video and monitor are not necessary, they are useful for counting otolith increments. The method demands precision and therefore requires individuals who can tolerate tedious tasks.

We experienced some limitations with the method. For example, we rarely could locate a release mark or resolve daily first-year growth increments on otoliths of age-1 kokanees by using the same grinding and mounting techniques used for age-0 fish.

The reliability of a release mark is not necessarily universal for all species of fish. Alternative marking methods such as hatchery temperature manipulation, which has been used to code otoliths of lake trout *Salvelinus namaycush* (Bergstedt et al. 1990) and Pacific salmon *Oncorhynchus* spp. (Volk et al. 1990) may be more appropriate

TABLE 4.-Bonferroni pairwise comparisons of number of otolith increments for age-0 kokanees stocked at various sites in Lake Pend Oreille, 1988-1989. Values are probabilities that difference = 0 between paired release groups.

Release group	Clark Fork early	Open water early	Clark Fork late	Sullivan Springs
Open water early	0.000			
Clark Fork late	0.028	0.084		
Sullivan Springs	0.058	0.019	1.000	
Open water late	0.002	0.118[a]	1.000[a]	1.000[a]
Mean difference	0.800	0.330	0.025	0.040

[a] Values of 0.118 and 1.000 in this row do not differ significantly ($P > 0.05$).

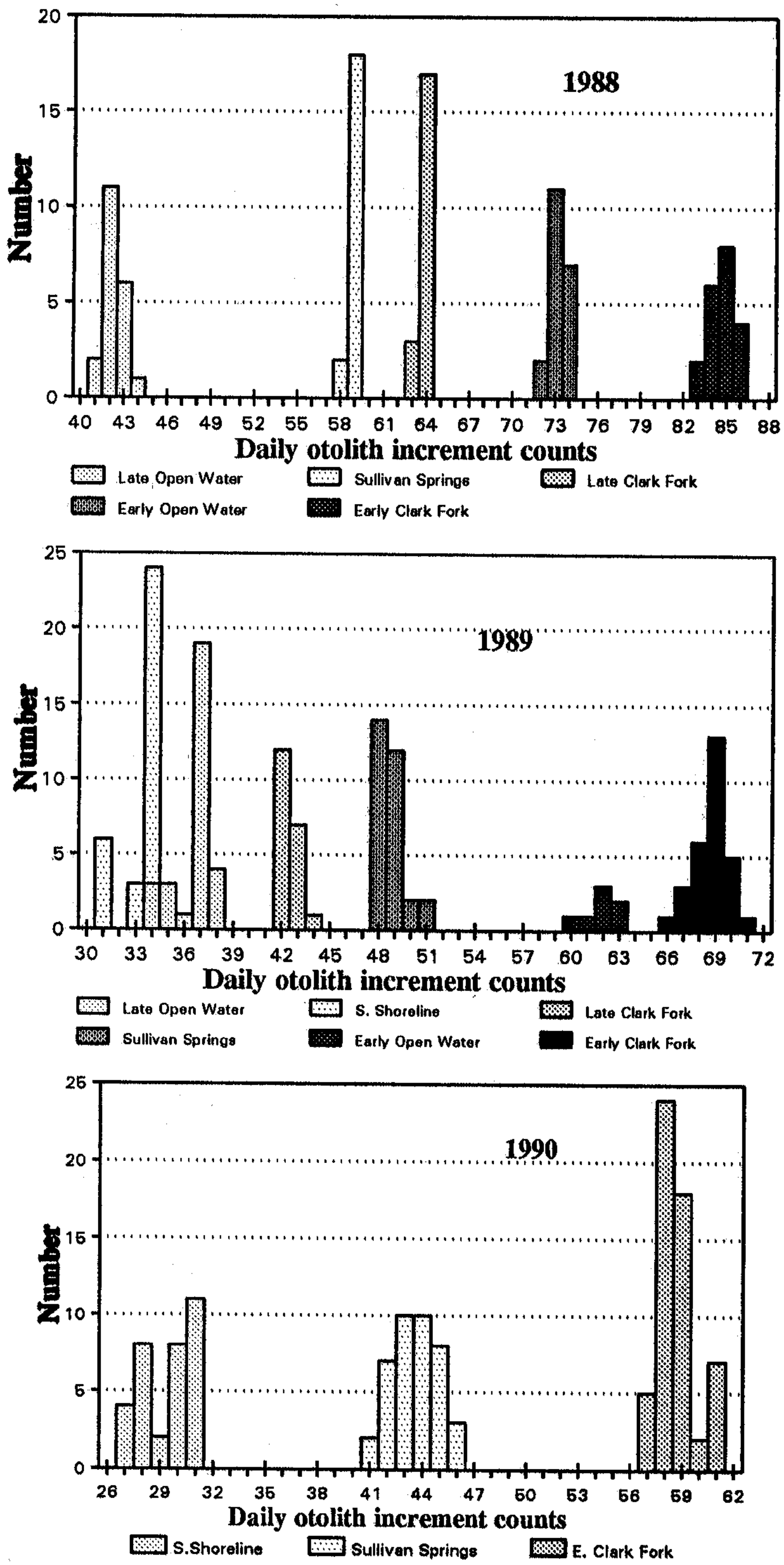

FIGURE 4. - Frequency distributions of daily Otolith increments formed by age-0 kokanees between the times they were released and recaptured in Lake Pend Oreille, 1988-1990. Release groups were identified by back-calculation to release dates.

for some species. If use of a release mark to identify hatchery fish seems plausible, it should be evaluated for each application. If daily otolith increments will be used to identify stocking groups, groups should be released at least 7 d apart.

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